Running headline: Compositional and functional indices for biodiversity monitoring.

Using compositional and functional indices for biodiversity conservation monitoring of semi-natural grasslands

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ABSTRACT

Compositional indicators (i.e. indices that focus on the identity of species, genes or phylogeny) have beenwidely used to estimate and monitor biodiversity, however, their use in combination with species and/orcommunity functional characteristics remains limited. Using large-scale, spatio-temporal data, we use both compositional and functional indices to investigate land-use change impacts on the vegetation of a semi-natural grassland ecosystem (Machair) for fourteen regions in Scotland, UK. Our study aimed to identify national- and regional-scale temporal vegetation patterns, and through use of simple compositional and functional indices (e.g. Competitor, Stress, Ruderal and Ellenberg scores) link observed changes to agricultural intensification and/or land-use abandonment. Using linear-mixed modelling and nonmetric multi-dimensional scaling, we showed significant national and regional-scale changes in species composition over time. Increases in diversity, particularly gains in Machair grassland, identified several regions that may have benefited from past government incentivised schemes to protect the Machair, but which may also be suffering from an extinction lag. Shifts in plant functional signatures (CSR & Ellenberg values) identified varying degrees of internal (competition) and external (land-use) factors, highlighting several regions where biodiversity change could be linked to reduced disturbance (i.e. lower grazing intensity) or greater disturbance (i.e. land-use intensification). Our results demonstrate the utility of simple compositional and functional indices for monitoring biodiversity of semi-natural grasslands and identifying land-use drivers of change across different spatial scales.

1. INTRODUCTION

Semi-natural grasslands (SNGs) constitute one of the most species-diverse communities in the World at small spatial-scales (Wilson et al. 2012). In Europe, the high level of biodiversity, characteristic of SNGs, is closely linked to century long associations with agricultural land-use (Eriksson et al. 2002; Poschlod and WallisDeVries 2002). However, for these tightly coupled social-ecological systems, cessation of traditional management (e.g. extensive grazing, hay making and rotational arable agriculture) in favour of land-use intensification or complete cessation of any form of management has become a widespread occurrence (refs). Typically linked to societal changes in response to economic factors, such land-use changes are considered the principal cause of habitat deterioration, fragmentation and biodiversity loss among European agricultural landscapes (Benton et al. 2003; Lindborg et al. 2008). Therefore, given current and forecasted global biodiversity loss (Sala et al. 2000), appropriate design and implementation of conservation management for attempts to maintain and enhance these systems has global as well as local and regional importance.

However, there is a growing concern regarding the effectiveness of current monitoring frameworks for attempts to conserve and enhance biological diversity (Lindenmayer and Likens 2010), particularly of semi-natural landscapes (de Bello et al. 2010). For semi-natural grasslands, one part of the problem is likely to be the broad spatial grain common to many large-scale monitoring studies (e.g. The Countryside Survey of Great Britain; http:// www.countrysidesurvey.org.uk/). European SNGs typically exhibit high spatial heterogeneity, usually as a result of spatially and temporally structured variation in agricultural management (Plieninger et al. 2006). Moreover, variation is intensified where land is apportioned to multiple landowners. Although such heterogeneity directly benefits the biodiversity of SNGs (Öster et al. 2007) and surrounding agricultural landscapes (Blitzer et al 2012), patterns of biodiversity change as a result of changing land-use (i.e. intensification vs abandonment) become extremely variable across relatively small spatial scales. As a result, management and

64 conservation policies designed and applied across large spatial extents may not always be
65 appropriate (Fortin and Dale 2005), often failing to be successfully translated across highly
66 heterogeneous landscapes (Stenseke 2006).

67 Another part of the problem is the complexity of biological diversity and the suite of
68 indicators designed to measure its various facets. This issue was recently addressed by de Bello
69 et al. (2010) who derived a framework to help standardise and converge the use of biotic
70 indicators (i.e. direct measures of components of biodiversity) and biotic drivers (i.e. factors
71 that govern local biodiversity) for monitoring biodiversity change, specific to semi-natural
72 habitats. Biotic indices (e.g. species richness, Shannon diversity, Simpson diversity, see
73 Magurran 2004) are commonly applied to measures of alpha diversity (*sensu* Whittaker 1960,
74 1972) or a subset of alpha, targeted at indicator species (i.e. suites of species that commonly
75 occur in a habitat under different environmental conditions – e.g. grazed or un-grazed,
76 favourable or non-favourable conservation condition etc; see Rosenthal 2003; Wittig et al.
77 2006). Such compositional measures are relatively easy to calculate and understand (Lamb et
78 al. 2009) but they fail to provide information of potential abiotic or biotic drivers governing
79 observed changes. On the other hand, direct measures of biotic drivers (e.g. pH, organic matter
80 and nitrogen content), for which links with biodiversity have been well researched (Hartley et
81 al. 2003; Pärtel et al 2004), are laborious and cost intensive, and therefore often unavailable.
82 This is especially true for large-scale spatial temporal monitoring studies.

83 To overcome these limitations, one approach is to use biotic indicators as surrogates for
84 biotic drivers, particularly those that focus on the functional characteristics of species and
85 ecological communities (i.e. functional indicators *sensu* de Bello et al. 2010). It is well
86 understood that plant functional characteristics, pre-adapted to the local environment, provide
87 distinct signatures to drivers of change that are not always easily measurable, and which often
88 relate well to land-use processes (Pakeman 2004; Quetier et al. 2007; Lewis et al. 2013). For
89 example: the Competitor-Stress-Ruderal (CSR) classification of plant functional types (Grime

1974) is frequently used to describe variation in vegetation through its functional composition (Cerabolini et al. 2010; Bornhofen et al. 2011). Among SNGs, shifts in CSR functional strategies can be observed. Land-use abandonment leads to size asymmetric competition for light (Mason et al 2011; Laliberte et al 2013), resulting in competitive displacement of grazing tolerant specialists (Tilman and Lehman 2001). Alternatively, declines in stress tolerant plant species should be observed with soil nutrient enrichment brought through land-use intensification (Maskell et al. 2010). Similarly, Ellenberg indicator values for plants (Ellenberg 1988), which define species habitat preferences and distribution, provide clear linkages between plant species occurrences and the environment, proving valuable for detecting changes in species compositions as a result of changes in land-use management. For example: Ellenberg N scores prove to be good correlates of mineralisable nitrogen (Rowe et al. 2011) and grassland productivity (Wagner et al. 2007).

Understanding the nature of vegetation change over time in this way can be paramount to the appropriate design of nature conservation practices, policies and land management across local, regional and national scales for all terrestrial habitats, not least SNGs. Coupled with monitoring indicator species, functional classifications such as plant CSR strategies, and/or Ellenberg values can prove extremely useful for highlighting the effects of land-use impacts.

In this study we demonstrate the potential utility of simple functional indicators as proxies of biodiversity drivers in indentifying temporal shifts in land use patterns among SNGs. Using archived biological records and revisitation data, valuable for quantifying long-term patterns of vegetation change (e.g. McCollin et al. 2000; Bennie et al. 2006; McGovern et al. 2011), we quantify national- and regional-scale temporal shifts in plant species diversity and composition for an internationally important SNG (machair). Through utilising simple land-use measures and functional indicators, surrogates for abiotic/biotic drivers, we link changes in the vegetation to changes in land-use, and identify regions where machair SNGs are subject to

116 land-use intensification and those that are subject to management declines over the last three
117 decades. We highlight regions of potential conservation concern and discuss how simple
118 environmental surrogates can be a useful, cost effective measure for multi-scale biodiversity
119 monitoring.
120
121 **2. METHODS**
122 **2.1 Study Area**
123 We used the grassland vegetation of the Scottish Machair as a case study (Fig. 1).
124 Confined globally to the northwestern fringes of Europe, sampled were14 regions,
125 encompassing almost the entire national resource of Scottish machair, i.e. over two thirds of
126 the World's total machair extent (ca. 30,000ha; Dargie 2000). Machair SNGs make a good
127 study system as their high floristic diversity and conservation value is tightly coupled to
128 century-long associations with human settlement (Gilbertson et al. 1996) and land-use
129 practices (Supporting Information S1), and presents a clear example of how SNGs are
130 threatened through agricultural and socio-economic change (Pakeman et al. 2012).
131
132 **2.2 Floristic Data**
133 Species compositional data were collated from two sample periods. Baseline data was
134 taken from the Scottish Coastal Survey (1975-1977; Shaw et al. 1983). The temporal aspect
135 was completed through a partial re-survey between 2009 and 2010 of regions known to include
136 machair communities, i.e. all transient communities from seaward embryo dunes to inland sand
137 affected peatlands, including machair grasslands (Angus 2006; Supporting Information S2).
138 Re-survey methodology followed closely that of the original survey (for details see Shaw et al.
139 1983), estimating cover of all higher plants and measuring environmental proxies for land-use
140 management within 5 m x 5 m plots.

Data specific to the focal community (i.e. machair grassland) was abstracted from the temporal dataset. Only those plots with a National Vegetation Classification (NVC) representative of machair grassland (Angus 2006; Table S1) were analysed. The resulting matrix (Matrix 1) comprised 321 species from 853 plots, from two sample periods. Matrix 1 was used to quantify biodiversity change. To reduce noise within the dataset (see Gauch 1982) Matrix 1 was further constrained, removing species present in less than 5 plots. This species data matrix (Matrix 2) comprised 213 species from 853 plots, from two sample periods and was used to quantify compositional change. A schematic representation of how the data was handled prior to analyses is provided in Supporting Information (Fig. S1).

2.3 Environmental Variables

Environmental proxies for land-use management included measures of past and present management, current grazing intensity, presence/absence of grazing animals and categorical measures of sward height for grasses and herbs (Table 1). Methods on how grazing pressure levels and past cultivation were determined are detailed in Supporting Information (S3).

2.4 Statistical Analysis

All statistical analyses were carried out using the R statistical software (Version 2.15.0, R Development Core Team 2012). Where necessary, data were transformed to meet assumptions of normality and homogeneity of variance.

2.4.1 Quantifying temporal change in biodiversity

Two biotic diversity indices: i) richness (i.e. the count of all species per 5m x 5m plot) and ii) the reciprocal of the Simpson's index (1/D) were calculated from Matrix 1. Paired *t*-tests, and General Linear Mixed Models (GLMM) with a poisson link function, were used to

identify statistically significant national- and regional-scale shifts respectively. The GLMM (M1) took the following structure:

(*response ~ year/region*, random = land-owner/plot) (M1)

Where, 'year' and 'region' were nested fixed factors, and land-owner and individual plots formed nested random factors accounting for the expected spatial and temporal auto-correlation.

These analyses were extended to investigate subsets of species, indicative of different vegetation conditions of the machairs taken from published guidance for conservation monitoring (Joint Nature Conservation Committee 2004; Table S2). Selected were four species indicator groups, indicative of good and poor quality machair grassland, fallow areas (indicative of continuous rotational arable agriculture) and active cultivation. Statistically significant shifts in mean species richness was tested using GLMM with a poisson link function. Similarly, temporal change in relative abundance was also investigated using linear mixed models with a residual maximum likelihood (REML) estimating procedure. Model structures equalled M1.

2.4.2 Quantifying temporal change in species composition

Non-metric Multi-Dimensional Scaling (NMDS; Legendre and Legendre 1998) of plots in two-dimensional ordination space was applied to Matrix 2, following the procedure recommended by Minchin (1987). The scores of the resulting NMDS axes were subsequently used as numeric values representing community composition in consecutive analyses (Kahmen et al. 2005). The Bray-Curtis (Bray and Curtis 1957) dissimilarity index was used for the computation of NMDS. Species contributing most to the overall Bray-Curtis measured temporal dissimilarity were identified through similarity percentage analyses (SIMPER; Clarke 1993). NMDS was performed using the function 'metaMDS' within the *vegan* package (Oksanen et al. 2013).

192 Temporal change in species compositions was measured by calculating the paired
193 distance between NMDS site scores for each plot for each survey year, in two-dimensional
194 ordination space. The statistical significance of these shifts provides a reliable indication of
195 species compositional change: paired t-test (national-scale) and linear-mixed modelling with
196 REML (M1; regional-scale).
197
198 *2.4.3 Linking environmental surrogates to biodiversity and compositional change*
199 Environmental surrogates, proxies for land-sue change (Table 1), and measured
200 functional indicators were projected onto the NMDS ordination, linking national and regional
201 compositional change with changing land-use parameters. Significant national and regional
202 shifts between survey years for the two functional classifications: CSR and Ellenberg scores
203 were tested using paired t-test (national-scale) and linear-mixed modelling with REML (M1;
204 regional-scale). Species abundances' (Matrix 2) were summarised as compositions of these
205 functional classifications (McGovern et al. 2011), acting as environmental surrogates for
206 various land-use changes. CSR scores of Grime (1974), derived using a spreadsheet based tool,
207 described in Hunt et al. (2004) and freely downloadable, were used to provide an indication of
208 external factors likely to alter plant growth (e.g. stress through lack of plant available resources
209 and disturbance). Similarly, Ellenberg indicator values, cover weighted and adjusted for British
210 plants, taken from Hill et al. (1999), were used to reflect plant species preferences in terms of
211 nutrient level (EbN), acidity (EbR), moisture (EbW), light (EbL) and salinity (EbS).
212 Land-use disturbance surrogates (Table 1) were examined to see how well they
213 explained the temporal compositional shifts. Between year differences in land-use measures
214 were regressed against between year differences in NMDS scores through further GLMMs:
215 (*response ~ Land-use*, random = land-owner/plot) (M2)
216
217 **3. RESULTS**

218 **3.1. Biodiversity change**

219 National gains in biodiversity, measured through both mean species richness (SR) and
220 Simpson's diversity (Sim) were significant (Table 2). Regional-scale analyses identified
221 significant gains in SR and Sim for two regions (Colonsay and Tiree), and significant declines
222 in SR and Sim for three regions (Benbecula, Harris and Monachs. Significant gains in SR
223 coupled with significant declines in Sim were observed for two regions (Barra and S.Uist).
224 Analyses of species subsets indicative of different vegetative states identified
225 significant national gains in richness and cover of machair grassland specialists, and a
226 significant national decline in relative abundance of species indicative of arable agriculture
227 (Fig. 2). Regional-scale analyses differentiated between regions in favourable condition, where
228 significant temporal gains in machair specialists occurred (Barra, Coll, Colonsay, Islay, S. Uist
229 and Tiree), and those of conservation concern where significant temporal declines in richness
230 and cover of species indicative of good quality machair grassland occurred (Benbecula,
231 Monachs and Shetland; Fig.2). Significant declines in the number of species indicative of
232 arable and rotational arable agriculture occurred among 5 regions (Barra, Harris, Monachs,
233 North Coast and North Usit), with significant declines in cover for 7 regions (Barra,
234 Benbecula, Islay, Monachs, North Uist, Shetland and South Uist).
235
236 **3.2 Compositional change**

237 The NMDS ordination showed clear shifts in species composition between survey years
238 (Fig. 3). This was supported by statistically significant temporal shifts in ordination scores at
239 both national- and regional-scales (Fig 4). Projection of environmental proxies identified a
240 positive shift along NMDS Axis 1 to indicate plant compositional change towards shorter
241 grassy and herbaceous swards. In addition, functional classifications Ellenberg Water and
242 Ellenberg Reaction were positively ranked with NMDS Axis 1 indicative of communities'
243 subject to higher saturation and more acidic substrate. Regional scale analyses identified five

regions to display significant positive shifts (Coll, Colonsay, Islay, S.Uist and Tiree) and one region to display a significant negative shift (Benbecula) along NMDS Axis 1 (Fig. 4).

Proxies for land-use disturbance, e.g. vegetation sward heights, evidence of livestock and cultivation, as well as stress tolerant species, were all strong correlates with NMDS Axis 2 (Fig. 3). Patterns indicate communities positively ranked with Axis 2 to have associations with cultivation practice, be it, past or present, and or low intensity rotational arable agriculture or larger-scale farming. Higher Ellenberg N values were also positively ranked with Axis 2. Only one region (Shetland) was found to positively and significantly shift along Axis 2 (Fig 4).

An opposite trend, where communities were negatively ranked with Axis 2 displayed strong associations with proxies for disturbance through grazing (i.e. shorter vegetation sward heights, heavy and moderate gazing, presence of sheep and species with higher stress tolerance (Figs 3). Significant negative shifts along this Axis were recorded for 2 regions (Barra and Coll; Fig. 4).

3.3. Linking environmental surrogates to temporal change

Significant national and regional shifts were indentified among the functional indicators. At the national-scale, a significant increase in functionally competitive species ($p < 0.001$) with a concomitant significant decline in ruderal species ($p = 0.005$) was observed (Fig. 5i). Eight regions mirrored this national trend, while only two (Barra and North Uist) were found to have statistically significant gains and declines in competitor and ruderal species respectively (Fig. 5i). Patterns not borne at the national-scale were revealed at the regional level. Three of the four regions (Barra, Monachs and North Uist) displaying significant declines in ruderal species were met by significant increases in stress tolerators. The opposing pattern where ruderals increased and stress tolerators declined was observed for five regions, with statistically significant change identified for two (Islay and Shetland). All significant changes in competitors were positive.

Significant national and regional shifts in Ellenberg values were also observed (Fig. 5ii). At the national-scale community composition shifted significantly towards species favouring acidic substrate (EbR, $p < 0.001$) and mineralisable nitrogen (EbN, $p = 0.008$), while species favouring moist soil conditions significantly declined (EbW, $p < 0.001$). At the regional-scale, significant changes were observed among 11 of the 14 regions surveyed (Fig. 5ii). Significant regional trends for EbR, EbN and EbW were consistent with the national changes observed, the only exception being a significant declines in species favouring mineralisable nitrogen for one region (Lewis). A general decline was observed in light competitive species (EbL), with the exception of one region (Monachs) where EbL significantly increased ($p < 0.001$). Significant shifts among salt tolerant species (EbS) varied across regions, significantly increasing (Harris, Monachs and Sanday), and significantly declining (Lewis, North Coast and Tiree).

Temporal change in measured land-use proxies indicative of arable agriculture (RecCult & OldCult) displayed a significant positive relationship with temporal compositional change measured as the change in NMDS scores for both Axis 1 and 2 (Table 3). Change in vegetation height classifications for graminoids over 50cm tall significantly and negatively related to compositional changes measured as the change in NMDS scores for Axis 1. Temporal change in the herb layers classifications i.e. Hrb<20 & Hrb>50 were also shown to have a significant positive relationship with species compositional change for Axis 1 and Axis 2 respectively (Table 3).

4. DISCUSSION

4.1. Biodiversity change: linking shifts to species indicator groups

The biodiversity value of semi-natural grasslands is well understood to be promoted by low-intensity land-use management (Eriksson et al. 2002; Poschlod and WallisDeVries 2002). For machair grasslands, century-long associations with human land-use (Gilbertson et al. 1996)

296 have resulted in these habitats developing in tandem, with a constant disturbance regime that
297 can now be considered a 'natural part' of the local habitat conditions. Given this tight historical
298 coupling between the biodiversity value and land-use practices, temporal changes in
299 biodiversity are likely to be linked to changes in the form, frequency and intensity of land-use
300 disturbances. In this study, we report significant national and regional temporal changes in
301 plant species diversity of Scottish machair grasslands. For many regions biodiversity
302 significantly increased, coupled with increases in richness and relative abundance of machair
303 grassland specialists. These positive changes in diversity were, for many regions linked to
304 concomitant gains in plant species indicative of rotational arable agriculture. Collectively these
305 species can be viewed as favourable machair species, i.e. species that belong to the habitat
306 specific species pool. A high proportion of these species can suggest a community to be more
307 complete, relative to the species pool (Pärtel et al. 2013). Results suggest for those regions
308 where biodiversity in favourable species increased, land-use management may be more in-line
309 with biodiversity conservation objectives, and therefore be regions of conservation importance.
310 Conversely, declines in favourable species, reported among regions where overall biodiversity
311 declined, highlights regions that may benefit from biodiversity restoration.

312 It is possible for positive changes to have been borne, in part by the adoption of
313 traditional management funded by the Environmentally Sensitive Areas (ESA) scheme.
314 Introduced to Scotland, in 1987, ESA schemes provided financial incentives to land managers
315 to undertake environmentally friendly practices to maintain and enhance biodiversity,
316 landscape and historic values of designated habitats. Although the schemes have since been
317 superseded, for several regions where they were implemented, species richness increased (Fig.
318 6). The pattern suggests that financially incentivised, low intensity management may have
319 either; directly or indirectly begun a process of widespread maintenance and enhancement of
320 the machair.

There is however an exception to this pattern as more northern ESA designated areas (Benbecula, North Uist and Shetland), displayed significant declines in favourable species. This raises debate over the appropriateness of preservation policies that specifically aim to preserve traditional management by making them financially attractive. In the EU, such policies are implemented through the Common Agricultural Policy (CAP, European Commission for Agriculture and Rural Development 2010), and their application is widely unquestioned. However, there are concerns that such artificial ties between people and the landscape will only lead to a decoupling between ecosystems and social systems (Fischer et al. 2012). Over time, naturally beneficial feedbacks between nature management and society are forgotten, and where monetary incentives cease so do the environmentally beneficial land-use practices. Biodiversity declines reported in this study for regions once designated as ESAs may then have rapidly reverted towards more economic, yet environmentally insensitive land-use practices.

Biodiversity gains alone may not necessarily be enough to conclude current land-use trends to be inline with biodiversity conservation objectives. Many regions where favourable species increased, species not belonging to the machair species pool (i.e. non-favourable species) increased. This pattern was significant for three regions (Coll, Islay and Tiree), for which species indicative of poor quality machair and active cultivation also increased. Results suggest, for these regions arable cultivation may have intensified supporting the finding of Lewis et al. (2013), where declines in functional richness were subsequently linked to land-use intensification.

Changes in the frequency and or intensity of disturbances can result in a relaxation in the competitive interactions among plant communities, permitting successful invasions of species from the regional species pool (Mayfield et al. 2010). Where disturbances are intensified, invaders commonly possess ruderality or opportunistic life strategy traits, and often do not belong to the invading habitat species pool. Equally, many extant species, particularly

long-lived pants will possess high inertia to environmental change, capable of persisting in a community as remnant populations long after changes have occurred (Diamond 1972; Eriksson 1996; Helm et al. 2006; Lindborg 2007). Therefore, at the regional-scale, invasions may not result in an immediate loss of favourable species from the habitat-specific species pool. The result is a colonization credit persisting of non-favourable species and a potential extinction debt (i.e. extant species expected to go extinct because threshold conditions for their persistence are no longer satisfied; Tilman et al. 1994) persisting of favourable species. Extinction debt has significant implications for conservation and restoration of threatened grassland habitats and species (Helm et al. 2006; Cousins 2009). Machair regions where biodiversity declines are not evident, but do show evidence of increased non-favourable species linked to land-use intensification should therefore be of high restoration importance, and warrant further investigations before desired parts of diversity are lost.

4.2. Compositional change: Linking shifts to environmental surrogates

For grassland communities, functional characteristics of plant species can help to distinguish between different land-use disturbances (Garnier et al. 2007, Pakeman 2011) and land-use changes (Lavorel and Garnier 2002; Lewis et al. 2013). In this study we use very simple functional classifications that differentiate between competitors, ruderals, stress tolerators (Grime 1974), as well as heliophyte, helophyte, halophyte, nitrophilous, ericaceous and calcicole species (Ellenberg 1988). Projection of these environmental surrogates on the NMDS ordination, coupled with the simple proxy measures of disturbance, provides an indication of land-use drivers of plant compositional change. We found significant national and regional compositional changes, measured as shifts in Bray Curtis dissimilarity (Bray and Curtis 1957) between survey years, suggesting a change in species community dynamics over time. Positive changes along NMDS Axis 1 scores, for which was the most common significant pattern among regions, could be linked to gains in ruderal species. As mentioned

earlier, invasion of ruderals is often a result of a decline in competitive interactions, usually attributable to some form of disturbance. Three regions (Coll, Islay and Tiree), where positive shifts along NMDS Axis 1 were identified, are also those regions identified in the biodiversity-change analyses to have likely suffered from agricultural intensification since the original survey. Although not clear from the NMDS ordination, regression of change in land-use proxies with change in ordination scores confirm arable cultivation practices to significantly drive compositional change along NMDS Axis 1, and therefore subsequently linked to significant compositional turnover of Scottish machair grasslands.

Correlates with NMDS Axis 2 were shown to be environmental surrogates linked to proxies of disturbances. Livestock grazing was negatively ranked, while cultivation and fertilisation practices were positively ranked. Land-use intensification through agricultural intensification, particularly in conjunction with nitrogen deposition typically results in compositional and taxonomic turnover among species assemblages, as environmental conditions change to favour more competitive, fast growing species that locally displace slow growing stress tolerators (Ordonez et al. 2009; Maskel et al. 2010). This pattern was evident for one region (Shetland) that displayed a significant positive shift along NMDS Axis 2, a significant decrease in stress tolerator species in favour of competitors and a significant gain in Ellenberg N, indicative of an increase in fertility. The national increase in Ellenberg N, is consistent with other long-term studies which report nation-wide gains in fertility values among semi-natural vegetation types across much of the UK (McCollin et al 2000; Smart et al. 2005). Experimental studies on calcareous grasslands have found N deposition to lead to shifts in species composition, with increases in rank grasses and loss of forbs and bryophytes (Morecroft et al., 1994; Lee and Caporn, 1998; Johnson et al. 1999). However, regional gains in Ellenberg N in this study were often shown with gains in competitive species without biodiversity loss. It may be regions are experiencing a relaxation period between land-use changes and stable equilibriums (Diamond 1972), whereby local extinctions are likely for those

399 species with lower soil nitrogen preferences (Sarr et al 2012). However, it may equally be due
400 to interacting effects of multiple land-use practices, arable agriculture and livestock grazing,
401 whereby grazers prevent potential dominants taking advantage of the increases in fertility.
402 Increased fertility among semi-natural grasslands therefore present concerns for
403 biodiversity conservation, particularly where land-use disturbances through traditional farming
404 practices are in decline. Declines in active management, particularly in grazing intensity,
405 typically result in increased dominance of competitor species and decline in ruderal species as
406 competition for space, light and nutrients increase (Pakeman 2004). For many European
407 countries, cessation in active management is recognised as the major threat to semi-natural
408 grasslands (Levin 2012). Evidence of management declines were certainly prominent in our
409 study, illustrating where shifts in functional indicators suggest reduced disturbance, these
410 regions were mostly those with significant declines in biodiversity. This highlights the negative
411 impact declines in active management and disturbance intensity have upon the conservation
412 value of semi-natural grasslands. For the machairs of Scotland, it appears a complete de-
413 coupling between the land and land-users to have a more immediate impact on biodiversity
414 value, than land-use intensification, stressing the importance of maintaining strong linkages
415 between nature and societies. Therefore, among landscapes with historic land-use traditions a
416 vital goal of conservation policy should be to help reconnect people with nature, fostering
417 socio-economic development while maintaining biodiversity (Fischer et al. 2012).
418

419 **4.3. Value of biotic indicators and environmental surrogates for biodiversity monitoring**

420 Given high temporal variability in land-use changes, and the ability of many species to
421 persist long after disturbances have occurred, biodiversity indices alones reveal little
422 information on whether current land-uses are in-line with conservation efforts. Interpretation of
423 results becomes more spurious where sample efforts are small and temporal lags are large.
424 However, introducing simple environmental surrogates as an additional component to

425 traditional diversity indices and select target species, help significantly for attributing land-use
426 drivers to observed patterns of change. Although rare, good quality temporal datasets can
427 provide documented evidence of vegetation composition prior to major global change impacts,
428 and are consistently proven for quantifying long-term patterns of vegetation change (McCollin
429 et al. 2000; Smart et al. 2005; Bennie et al. 2006). Given the extensive sampling effort in this
430 study non-representative samples should not have undue influence, however we do
431 acknowledge that caution should be expressed in the interpretation from only two snapshots in
432 time, particularly with large temporal lags between surveys.

433 This study clearly demonstrates the value of simple biotic indicators and environmental
434 surrogates for use in biodiversity monitoring assessments, demonstrated here across a large
435 national extent of semi-natural grasslands. In this study we used very simple indices and
436 functional indicators, which allowed us to differentiate between possible regions and infer with
437 some confidence the likely land-use changes driving species compositional and biodiversity
438 patterns. Our approach shows that functional indicators need not be overly complex,
439 particularly for assessing broad-scale regional change. Functional traits measures that are
440 nowadays also easily accessible are exceptional useful for gaining in depth knowledge of land-
441 use impacts on the composition and biodiversity of plant assemblages, and can also be used to
442 identify community assemble processes (refs). Nevertheless, high variability in terms of land-
443 use practices, land-use change and species compositions within single regions complicate the
444 generalisations that can be made. Therefore, we argue that broad functional classifiers to be
445 equally proficient in identifying patterns and process of change at broader spatial scales, and
446 are a useful tool for identifying regions of conservation concern that may warrant more
447 detailed, finer-scaled investigations.

448 Scale therefore is an extremely important factor when making interpretations from
449 biodiversity monitoring studies. Many pattern and trends identified at regional-scales were not
450 apparent at the national scales, as contrasting divergent and convergent trends between

451 geographic regions counteract, revealing little or no national pattern. Regional results from this
452 study are still generalisations, and are unlikely to reflect entire grassland assemblages within a
453 predefined geographic boundary. Nevertheless, our results clearly show the value to analysing
454 national data at smaller geographic scales.
455
456 **5. Conclusions**
457 This study clearly demonstrates the value of simple biotic indicators and environmental
458 surrogates for use in biodiversity monitoring assessments, demonstrated here across a large
459 national extent of semi-natural grasslands. We used very simple indices and functional
460 indicators, which allowed us to differentiate between possible regions and infer with some
461 confidence the likely land-use changes driving species compositional and biodiversity patterns
462 of Scottish semi-natural coastal grasslands. Our approach highlights that functional indicators
463 need not be overly complex, particularly for assessing broad-scale regional change. Functional
464 traits measures that are nowadays relatively easily to obtain, are also exceptionally useful for
465 providing an in-depth understanding of land-use impacts on the composition of vegetation
466 (Pakeman et al., 2009; Lewis et al., 2014), as well as insights into the community assembly
467 processes that govern plant assemblages (e.g. Mouchet et al., 2010). Nevertheless, high
468 variability in terms of land-use practices, land-use change and species compositions within
469 single regions complicate the generalisations that can be made. Simple, broad functional
470 classifiers, as used here, can therefore be equally proficient in identifying patterns and process
471 of change at broader spatial scales, and are a useful tool for identifying areas of conservation
472 concern that may warrant more detailed, finer-scaled investigations.
473
474 Spatial extent is therefore an extremely important factor when making interpretations
475 from biodiversity monitoring studies. In this study, many patterns and trends identified at the
476 regional scale were not apparent at the larger national scale, as contrasting divergent and
477 convergent trends between geographic regions counteracted, resulting in little or no national
478 pattern. Regional results from this study are still generalisations, and are unlikely to reflect
479 entire grassland assemblages within a pre-defined geographic boundary. Nevertheless, our
480 results clearly showed the value of analysing
481
482
483
484

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486

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490

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683

684 **Table 1:** Description of land-use proxies measured for each 5m x 5m plot.

Disturbance Proxy	Data description	Environmental Variable	Abbreviation
Animal signs	Plot level presence or absence of dung from 5 herbivore species	Cattle	N/A
		Sheep	N/A
		Rabbits	N/A
Grazing pressure	Intensity of herbivore grazing pressure	None	NoGrz
		Light	LghtGrz
		Moderate	ModGrz
		Heavy	HvyGrz
Vegetation structure	Indication of vegetation height and structure for each plot (0,1,2,3 = no, sparse, intermediate and full cover respectively)	Grasses < 20cm	Gra20
		Grasses 20-50cm	Gra2050
		Grasses > 50cnm	Gra50
		Herbs < 20cm	Hrb20
		Herbs 20-50cm	Hrb2050
		Herbs > 50cnm	Hrb50
Cultivation	Plot level presence or absence of a suite of cultivation factors, from no cultivation to current standing crop	No Cultivation	NoCult
		Standing Crop	StdCrp
		Recent Cultivation (past 3 years)	RecCult
		Old Cultivation (4 or more years)	OldCult

685

686

Table 2. National and regional Mean ($\bar{x}$) temporal shift in the species diversity of machair grassland between 1976 and 2010 measured through i. richness (species count) and ii. Inverse Simpson index. Significance tested through paired t tests (national) and general liner mixed model with poisson function (regional). '***' $p \leq 0.001$, '**' $p \leq 0.01$, '*' $p \leq 0.05$.

	Species Richness			1/Simpsons		
	$\bar{x}$	z	sig	$\bar{x}$	z	sig
National	2.32	7.60	***	-0.25	2.17	*
Barra	4.21	4.31	***	-2.33	-4.28	***
Benbecula	-3.36	-1.99	*	-1.71	-1.97	*
Coll	10.07	12.51	***	0.67	1.72	
Colonsay	14.70	7.39	***	2.68	2.75	**
Harris	-5.13	-3.60	***	-2.44	-3.12	**
Islay	8.89	8.47	***	0.22	0.40	
Lewis	-2.15	-2.59	**	0.40	1.02	
Monach	-3.06	-2.87	**	-1.46	-2.97	**
North Coast	-1.02	-1.33		1.15	3.44	***
North Uist	-0.35	-0.68		-1.30	-5.16	***
Sanday	0.04	0.04		0.17	0.38	
Shetland	-3.29	-2.97	**	-1.08	-1.89	
South Uist	4.98	7.40	***	-0.93	-2.85	**
Tiree	10.23	13.96	***	1.96	5.34	***

Table 3. Significance of change in land-use disturbance proxies in explaining temporal compositional change as measured by the NMDS analyses (Figure 3). Significance tested through general liner mixed model. '***' $p \leq 0.001$, '**' $p \leq 0.01$, '*' $p \leq 0.05$.

	NMDS AXIS 1			NMDS AXIS 2		
Land-Use						
NoGrz	0.05	0.66		0.02	0.42	
LightGrz	0.05	0.76		0.03	0.77	
ModGrz	0.03	0.37		0.03	0.57	
HvyGrz	0.05	0.66		0.06	1.28	
RecCult	0.48	5.69	***	0.28	4.97	***
OldCult	0.17	3.02	**	0.11	3.11	**
StndCrp	0.10	0.68		0.08	0.83	
Gra20	-0.02	-1.08		<0.01	<0.01	
Gra2050	-0.01	-0.65		-0.01	-1.13	
Gra50	-0.06	-3.23	***	-0.01	-1.17	
Hrb20	0.04	2.16	*	0.01	0.86	
Hrb2050	0.03	1.65		<0.01	0.35	
Hrb50	0.06	1.67		0.05	2.31	*
Cattle	<0.01	0.03		0.02	1.07	
Sheep	0.01	0.30		-0.02	-1.00	
Rabbit	0.01	0.38		-0.01	-0.50	

716 **Fig. 1.** Distribution of original and resurveyed study sites across Scotland. For site details see
717 Appendix A.
718
719 **Fig. 2.** Mean richness and % cover change for species groups indicative of different conditions
720 and land-use states of machair grasslands: Cult = species indicative of cultivated land; Fallow
721 = species indicative of rotational arable machair i.e. fallow grasslands; Machair = species
722 indicative of machair grasslands; Poor = species indicative of degraded machair grasslands.
723 Mean differences are presented with standard error bars of the mean. Shaded bars represent
724 statistically significant change ($p < 0.05$) tested using General Linear Mixed Models (GLMM;
725 Richness) and linear-mixed modelling with residual maximum likelihood (REML;
726 Abundance).
727
728 **Fig. 3.** NMDS ordination of machair grassland communities across Scotland, computed using
729 the Bray Curtis dissimilarity. Figures i-ii: Displayed are the convex hulls for each survey
730 (dashed and solid lines refer to the original and resurvey respectively). (i) Projected
731 environmental proxies for land-use $p<0.001$ (see Table 1). (ii) Projected functional signatures:
732 Ellenberg and CSR scores. (iii) Displayed as text are species with a significant statistical
733 relationship ($p < 0.001$) and a goodness of fit ($r^2 \geq 0.05$) along Axis 1 and or Axis 2. Species
734 are represented by the following letter codes: Aa *Ammophila arenaria*, As *Agrostis stolonifera*,
735 Bry Bryophytes, Cf *Carex flacca*, Cn *Carex nigra*, Cp *Cardamine pratensis*, Er *Elymus repens*,
736 Gp *Galium palustre*, Gv Galium verum, Hv, *Hydrocotyle vulgaris*, Lp *Lolium perenne*, Pa
737 *Potentilla anserina*, Pv P*runella vulgaris*, Rr *Ranunculus repens*, Sp *Succisa pratensis*, Tp
738 *Thymus polytrichus*, Tr *Trifolium repens*. Non-significant species are represented by '•'.
739
740 **Fig. 4.** National and regional shifts in NMDS ordination axes scores for computed using Bray
741 Curtis in two dimensional ordination space. Displayed are kernel densities for NMDS axis 1

742 and axis 2. Dashed and solid lines refer to the original and resurvey respectively. Shaded
743 density graphs (green = original survey & purple = resurvey) represent statistically significant
744 shifts between survey years ($p < 0.05$) tested using nested linear mixed models with residual
745 maximum likelihood (REML).
746
747 **Fig. 5.** National and regional cover-weighted functional classification values (i. CSR & ii.
748 Ellenberg) for the surveyed machair grasslands of Scotland. Mean plot scores are presented
749 with standard error bars of the mean. Grey bars represent statistically significant change
750 between survey years ($p < 0.05$) tested using nested linear mixed models with residual
751 maximum likelihood (REML).
752
753 **Fig. 6.** Patterns between regional species richness gains and loses since 1976 and those regions
754 once designated as an Environmentally Sensitive Area (ESA).